

Application No. 10/559,784
Amendment dated September 15, 2009
Reply to Office Action of July 15, 2009

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REMARKS

Claims 1 - 6 and 11 - 14 have been withdrawn. Claims 9 - 10 have been canceled. Claims 7 - 8 are pending and are the subject of this Office Action. Support for claims 7 - 8 can be found throughout the specification including the Drawings and claims as filed originally. No new matter has been added.

Applicant respectfully reserve the right to pursue any non-elected, withdrawn, canceled or otherwise unclaimed subject matter in one or more continuation, continuation-in-part, or divisional applications.

It is submitted that the claims, herewith and as originally presented were in full compliance with the requirements of 35 U.S.C. § 112. The amendment of the claims, as presented herein, is not made for purposes of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112. Rather, this amendment is made simply for clarification and to round out the scope of protection to which Applicants are entitled. Furthermore, it is explicitly stated that the herewith amendment should not give rise to any estoppel.

Reconsideration and withdrawal of the rejections of this application in view of the amendments and remarks herewith, is respectfully requested, as the application is in condition for allowance.

Applicant now turns to comments made by the Examiner in this Office Action as follows.

Office Action

1. The rejection of claims 7-10, are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosaka et al. (Exp Cell Res 245: 245-251, 1998) in view of Haruta et al., (Nat Neurosc 4: 1163-1164, 2001), is applied to the amended claims 7-8, for reasons of record in the Office Action, dated 8 January 2009.

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The Examiner states, "Applicant's arguments are based on the current claim amendments. Specifically Applicant argues that the present invention as recited in amended claim 7 is drawn to the use of iris pigmented epithelial cells "not being subjected to a gene transfer", while the Haruta reference teaches differentiation into retinal nerve cells after a "Crx gene is transferred and forcibly expressed" in the iris-derived cells. Applicant also argues that Haruta teaches iris derived cells that are different from the claimed iris pigmented epithelial cells, because the cells of Haruta do not produce retinal photoreceptor cell marker rhodopsin, without the Crx gene transfer. Applicant concludes that the invention as currently amended in claim 7 is non-obvious over the prior art teachings because the skilled person would not accomplish the same results as claimed, without forcibly expressing the Crx gene in the iris-derived cells. As such the rejection is requested to be withdrawn.

Applicant's arguments are fully considered but not found to be persuasive, because arguments that rely on particular distinguishing features are not persuasive when those features are not recited in the claims. Applicant's arguments are largely directed to the Haruta reference, emphasizing the limitations inserted in the current claim amendments. As stated in the previous Office Action, Haruta et al teach the plating and maintenance of iris tissue from adult rats in serum free culture medium containing bFGF or FGF2, resulting in the proliferation of cells as a monolayer (Figure 1a, page 1163, para 2). Haruta et al. also teach that the iris derived cells are positive for a retinal ganglion cell marker, neurofilament 200, wherein such differentiation into a retinal nerve cell is accomplished without any gene transfer. The previous Office Action does not describe Figures 2 or 3, nor does it describe the differentiation to retinal photoreceptor cells from iris derived cells by transferring a Crx gene, as extensively argued by Applicant. Furthermore, the claims as amended do not require the differentiation to retinal photoreceptor cells, nor do they specifically recite that the retinal nerve cells are photoreceptor cells that produce rhodopsin. Therefore, Applicant is diverting the arguments to the newly amended subject matter, side-tracking the arguments presented in the previous Office Action.

The instant claims require a method for producing retinal nerve cells by isolating and differentiating iris pigmented epithelial cells, wherein the differentiation is induced by adherent culturing in a serum-free culture medium containing one of FGF2, FGF9 and CNTF. Based on the definition provided in the instant disclosure, a nerve cell can

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comprise neurons as well as non-neuronal or glial cells (para 0122, 0123), and a retinal nerve cell can comprise a retinal visual cell, bipolar cell, Muller glial cells, etc. (para 0040). Additionally iris-derived cells are broadly defined as comprising IPE cells, and Kosaka et al teach the isolation of IPE cells. It is to be noted that USPTO personnel are to give claims their broadest reasonable interpretation in light of the supporting disclosure. *In re Morris*, 127 F.3d 1048, 1054-55, 44 USPQ2d 1023, 1027-28 (Fed. Cir. 1997). Narrow limitation contained in the specification cannot be inferred in the claims where the elements not set forth in the claims are linchpin of patentability. See *In re Philips Industries, Inc. v. State Stove & Mfg. Co.*, 522 F.2d 1137, 186 USPQ 458 (CA6 1975), 237 PTJA A-12. While the claims are to be interpreted in light of the specification, it does not follow that limitations from the specification may be read into claims. On the contrary, claims must be interpreted as broadly as their terms reasonably allow. See *Ex parte Oetiker*, 23 USPQ2d 1641 (BPAI, 1992). Applicant is reminded that the claims define the subject matter of his invention and that the specification cannot be relied upon to read limitations into the claims.

In view of the above discussion, it is reiterated from the last Office Action that:

It would have been, therefore, obvious to the person of ordinary skill in the art at the time the claimed invention was made to modify the method of inducing differentiation of IPE cells to lens cells by adherent or monolayer culture method in medium containing serum as taught by Kosaka et al., to the monolayer culture in a serum free medium of Haruta et al., whereby the iris derived cells differentiate to retinal cells expressing neuronal antigen (i.e. inherently retinal nerve cells). The person of ordinary skill in the art would have been motivated because IPE and the neural retina have a common developmental origin, thereby giving rise to retinal neurons (Haruta et al. page 2163).

Because, the source of the cells and culture conditions in the prior art teachings and the currently claimed invention are the same, the claimed invention as a whole stands *prima facie* obvious over the combined teachings of the prior art and stay rejected."

Applicants respectfully disagree. The Examiner alleges that the Haruna reference teaches that the iris derived cells are positive for a retinal ganglion cell marker, neurofilament 200, wherein such differentiation into a retinal nerve cell is accomplished

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without any gene transfer.

However, neurofilament 200 is a protein molecule known to be expressed in many kinds of nerves. Accordingly, although the result of iris derived cells being positive for neurofilament 200 suggests that the iris derived cells are differentiated into nerve cells, the result does not indicate that the iris derived cells are differentiated into particular retinal nerve cells. In other words, neurofilament 200 expresses in retinal ganglion cells, but this does not indicate that cells in which neurofilament 200 expresses are necessarily retinal ganglion cells. In fact, Haruta et al. does not describe that iris derived cells are differentiated into retinal ganglion cells.

In view of the above, Applicant respectfully considers that the Examiner's allegation that Haruta et al. teaches iris derived cells are differentiated into retinal nerve cells without any gene transfer is incorrect.

The Examiner alleges that a person of ordinary skill in the art would have been motivated to differentiate iris pigmented epithelial cells (IPE cells) into retinal nerve cells because IPE cells and the neural retina have a common developmental origin, thereby giving rise to retinal neurons. However, at the time of filing of the present application, it was completely unknown whether IPE cells can be differentiated into retinal nerve cells regardless of the fact that IPE cells and retinal nerve cells have a common developmental origin. "*Biochem. Biophys. Res. Commun.*, 2004 March 26; 316(1), pgs. 1-5", a treatise written by the inventors of the present invention, reports the result of examining differentiation from human retinal pigment epithelial cells to neurons (this treatise is attached hereto). The treatise describes that although retinal pigment epithelial cells could be induced into neuron-like cells, it was difficult to induce the retinal pigment epithelial cells into particular retinal cells, and no cells positive for a photoreceptor cell marker was observed.

Applicant, in particular, believes that a conclusion of obviousness cannot be

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made in view of the U.S. Supreme Court's and the USPTO's current interpretation of obviousness under 35 U.S.C. § 103.

The PTO has issued Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 ("Guidelines") in view of the Supreme Court's recent decision in KSR International Co. v. Teleflex Inc., 550 U.S. ___, 82 USPQ2d 1385 (2007). The Guidelines were published in the Fed. Reg., Vol. 72, no. 195, October 10, 2007. As pointed out in the Guidelines, the Supreme Court in KSR reaffirmed the analytical framework for determining obviousness as set forth in Graham v. John Deere Co., 338 U.S. 1, 148 USPQ 459 (1966), and also held that the Federal Circuit's application of its teaching-suggestion-motivation test was too formalistic.

Under Graham, obviousness is a question of law based on underlying factual inquiries that address (1) the scope and content of the prior art, (2) the differences between the claimed invention, and (3) resolving the level of ordinary skill in the pertinent art. Consideration must also be given to secondary factors, such as, for example, evidence of commercial success, long felt but unsolved needs, failure of others, and unexpected results. The Supreme Court stated in KSR that "While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls." The Guidelines go on to state that "Once the *Graham* factual inquiries are resolved, Office personnel must determine whether the claimed invention would have been obvious to one of ordinary skill in the art."

The Guidelines proceed then to articulate seven independent rationales on which to properly base a rejection under 35 U.S.C. § 103: (1) combining prior art elements according to known methods to yield predictable results, (2) substitution of one known element for another to obtain predictable results, (3) use of known technique to improve similar devices, methods or products in the same way, i.e., to obtain predictable results, (4) applying a known technique to a known device, method or

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product ready for improvement to yield predictable results, (5) choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success, i.e., obvious to try, (6) evidence of design incentives or other market forces sufficient to prompt skilled artisan to vary prior art in a predictable manner to result in claimed invention, and (7) evidence of some teaching, suggestion, or motivation in the prior art that would have led the skilled artisan to modify or combine prior art to arrive at claimed invention, i.e., predictable modification. All of these tests have the requirement of predictability. That is lacking in the present case.

In view of the above, at the time of filing of the present application, a person of ordinary skill in the art could not **predict** that IPE cells can be differentiated into retinal nerve cells although IPE cells and retinal nerve cells have a common development origin. Accordingly, Applicant respectfully considers that the Examiner's allegation that a person of ordinary skill in the art would have been motivated to differentiate IPE cells into retinal nerve cells is incorrect.

Therefore, claim 7 directed to differentiation from IPE cells into retinal nerve cells is not obvious over Kosaka in view of Haruta. Consequently, claim 8 depending from claim 7 is not obvious over Kosaka in view of Haruta.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

CONCLUSION

Applicants submit that all claims are allowable as amended and respectfully request early favorable action by the Examiner. Applicant's representative would like to discuss this case with the Examiner to learn if any outstanding issues remain after consideration of this Amendment. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application,

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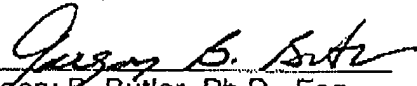
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the Examiner is cordially invited to call the undersigned attorney of record. Although it is not believed that any further fee is needed to consider this submission, the Office is hereby authorized to charge our deposit account 04-1105 should such fee be deemed necessary.

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Respectfully submitted,

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Adult human retinal pigment epithelial cells capable of differentiating into neurons

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Abstract

We investigated the ability of adult human RPE cells to differentiate into neurons. Two cell lines were evaluated. The cells were cultured in medium with 8% serum, transferred to a neural stem cell maintenance culture, and induced to differentiate with retinoic acid. The cells were immunocytochemically examined at each step. The cells showed epithelial-like morphology with 8% serum and all were immunoreactive for β -III tubulin. After transfer to the stem cell maintenance culture, they changed morphologically and became immunoreactive for MAP5 and neurofilament200 after induction with retinoic acid. The ratio of MAP5 positive cells was higher in the young adult RPE cell line. No GFAP or *rod*-opsin immunoreactive cells were observed. Adult human RPE cells, even from old person are capable of differentiating into neurons, although the ratio of mature neurons was greater in the young than in the old cell line in this condition.

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Stem cells of various tissues have recently gained interest because of their potential use as sources of cell transplantation. Furthermore, the possibility of transdifferentiation of stem cells even beyond germ layers has been reported, such as blood cell differentiation from neural stem cells and neuronal differentiation from hematopoietic cells [1], although this remains controversial [2].

Retinal progenitor cells are present in embryonic retinas [3] and are a potential retinal cell transplantation source [4]. It also has been shown that even in adult mice retinal neural progenitor cells reside in ciliary epithelium and can generate photoreceptors [5].

We previously reported that adult rat brain-derived neural stem cells could integrate and survive after transplantation into the retina [6] and that they differentiated

into neurons even in adult host retina, although they did not show any retinal neuronal marker expression [7].

We therefore investigated the potential of progenitor cells in other parts of the eyes such as embryonic retinal progenitor cells [8], adult rat iris-derived neural progenitor cells [9] and, in the study presented here, adult retinal pigment epithelium cells.

Early in the 1970s, the word 'transdifferentiation' was already used for the ability of retinal pigment epithelial cells to dedifferentiate to an immature status and redifferentiate into another type of cell [10]. Since then it has become well known that RPE cells of adult newts can transdifferentiate into lens epithelial cells. Moreover, these cells can completely regenerate the neural retina after its removal [11,12].

Retinal pigment epithelial (RPE) chick embryo cells also can transdifferentiate into neural cells with acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) [13].

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As for mammals, in the presence of bFGF, cultured early embryonic rat RPE cells that have not acquired pigment yet develop to form a retina-like multilayer structure containing neuronal cells that express markers of retinal ganglion, amacrine, and rod photoreceptor cells. However, the ability of rat RPE cells to transdifferentiate is restricted to a certain age range [14].

In this study, we investigated whether adult human RPE cells have the ability to differentiate into neurons and evaluated the difference between young adult RPE cells and the RPE cells of an elderly man.

Materials and methods

Human retinal pigment stem cells

Two human adult retinal pigment epithelial cell lines were used in this study, H80HRPE (80-year-old human differentiated pigmented epithelial cells)-6 and ARPE-19.

H80HRPE-6 was established by Eguchi et al. and harvested from the eye bank eye of an 80-year-old male, while ARPE-19 was harvested from a young eye.

Establishment of the human RPE cell clone

Eye cups were made from the eye, after which the neural retina was removed from the eye cups and placed in PBS. The eye cups were incubated in 0.05% EDTA, and only then the pigmented epithelial layer was peeled off. After trypsinization of the pigmented epithelium, dissociated cells were cultured in a collagen coated dish. Cells were passaged using trypsin EDTA, and after several passages the cells were cultured at low density and clones of the cells were obtained by means of colony rings. We used one clone that appeared healthy (H80HRPE-6) [12,15].

Monolayer cell culture

Epithelial culture. First, the cells of these two cell lines were cultured in Eagle's minimum essential medium (MEM; Gibco, Rockville, MD) supplemented with 8% heat inactivated fetal bovine serum, 1% penicillin-streptomycin, and, at 37°C, with 5% CO₂. The medium was changed every 3 days.

Stem cell culture. Next, we cultured the cells under the same condition as used for the maintenance of neural stem cells. They were cultured on laminin/poly-L-ornithine-coated dishes containing Dulbecco's modified Eagle's medium-Ham's F12 (DMEM/F12; Gibco) supplemented with N₂ (Gibco) 20 ng/ml basic fibroblast growth factor (bFGF; Genzyme, Cambridge, MA) and REC Human epidermal growth factor (EGF; Genzyme), and incubated at 37°C in humidified 5% CO₂ in air. The medium was changed every 3 days.

Differentiation culture. After having been cultured for more than 2 weeks under these conditions, the cells were induced to differentiate in the DMEM/F12 by the addition of N₂, 0.5% PBS and 0.5 nM retinoic acid for 10 days.

Immunocytochemistry. The monolayer culture cells were fixed in 4% paraformaldehyde (Wako Pure Chemical, Osaka, Japan) in PBS for 30 min at 4°C and blocked with 20% Block Ace (Dainihon-Seiyaku, Osaka, Japan) in PBS containing 0.005% saponin (Sigma, St. Louis, MO) for 30 min. After removal of the blocking solution, the cultured cells were incubated with primary antibodies for 60 min at room temperature. Primary antibodies were used at the following concentrations: mouse monoclonal anti-pancytokeratin (1:400; Sigma) as the retinal epithelium cell marker, rabbit polyclonal anti-ZO-1 (1:200;

Zyted Laboratories, South San Francisco, CA), as the tight junction marker, rabbit polyclonal anti-musubi (1:400; Chemicon) as central neural stem cell marker, mouse monoclonal anti- β tubulin isotype 1D (β -III tubulin; 1:500; Sigma), mouse monoclonal anti-microtubule associated protein (MAP) 5 (1:1000; Chemicon, Temecula, CA) and mouse monoclonal anti-neurofilament 200 (1:1000; Sigma) as neuronal markers, rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) as the glial cell marker, and mouse monoclonal anti-rod-opsin (RGT-PI, 1:10,000; Sigma) as the rod photoreceptor cell marker. After washing with PBS, the cells were incubated with the appropriate secondary antibodies for 60 min at room temperature. Secondary antibodies were used at the following concentrations: Alexa Fluor 488 goat anti-mouse immunoglobulin (Ig, 1:500; Molecular Probes, Eugene, OR) and Alexa Fluor 546 goat anti-mouse immunoglobulin (Ig, 1:500; Molecular Probes). Cell nuclei were counterstained with 4',6-diamino-2-phenylindole, dihydrochloride (DAPI) (1 μ M; Molecular Probes) added to the secondary antibody solution. All antibodies were diluted in PBS containing 0.005% saponin and 5% Block Ace. The cells were then washed with PBS and mounted with glycerol/PBS (1:1).

Results

Characteristics of human retinal pigment cell line

H80HRPE-6 cells and ARPE19 cells showed a flat and polygonal epithelial-like morphology without pigment in epithelial culture medium with fetal bovine serum (Fig. 1A). They had remained healthy after freezing and thawing. Cells of either line contained no visible pigment until after 5 months' culture, when some cells of ARPE19 showed visible pigment in their cytoplasm.

The cells cultured for 2 weeks in the epithelial culture medium were examined immunocytochemically. Most of the cells showed immunoreactivity for pancytokeratin, a marker for epithelial cells, and some parts of the cells for ZO-1, a marker for tight junction (Fig. 1B). Moreover, all the cells showed β -III tubulin (Fig. 1C), but no MAP5 positive cells were observed (Fig. 1D).

Culture under conditions used for neural stem cell maintenance

We cultured the adult human RPE cells in the MEM with fetal bovine serum for several weeks (epithelial culture) and then transferred them to serum-free medium with bFGF and EGF on laminin/poly-L-ornithine coated dishes (stem cell culture). The shape of the cell bodies of H80HRPE-6 changed to spherical or spindle-like after 2 months of culture (Fig. 2A). ARPE-19 cells became elongated after several days and then spherical or spindle-like.

In this condition we could not observe pancytokeratin, ZO-1, MAP5, nor NF200 positive cells.

Differentiation induced for 10 days with retinoic acid

After 10 days in a differentiation culture, the cells extended multiple processes into the medium with

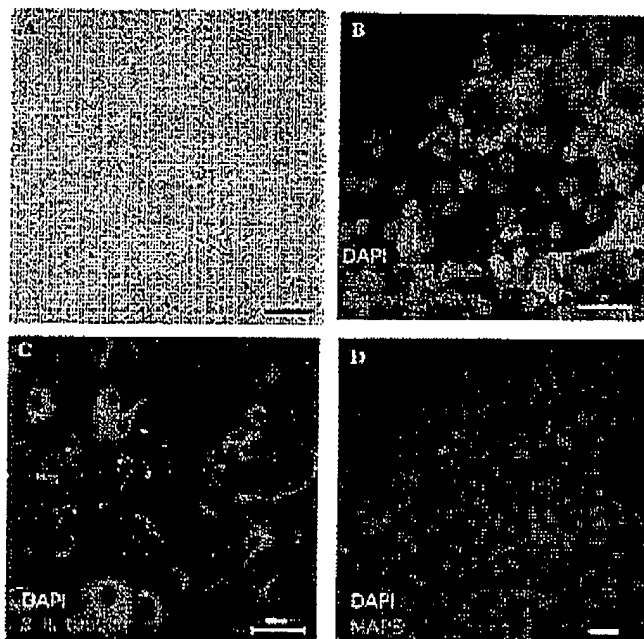


Fig. 1. RPE cells cultured in Eagle's minimum essential medium supplemented with 5% fetal bovine serum. (A) Phase contrast photograph. The cells show flat, epithelial-like morphology. The bar = 100 μ m. (B–D) Immunocytochemistry for pancytokeratin (B, green) and ZO-1 (B, red), for β -III tubulin (C, green), and for MAP5 (D, green). Nuclei were stained with DAPI. The bar = 50 μ m (B,C). The bar = 100 μ m (D).

retinoic acid. All the cells were β -III tubulin-positive (Fig. 2B), and a few cells in the H80HrPE-6 culture and some in the ARPE-19 culture showed immunoreactivity for MAP5 (Figs. 2C and D). Some of the cells were also NF200-positive (Fig. 2D). However, we could not detect any GFAP- or rhodopsin-positive cells (Figs. 2E and F), and even when we tried to induce glial differentiation with a high concentration of FBS, no GFAP-positive cells were observed in either cell line.

Discussion

The RPE cells of many vertebrate species can dedifferentiate and transdifferentiate in response to changes in their environment. It is well known that in amphibians RPE cells transdifferentiate not only into lens cells, but also into retinal neurons [10,11,16]. It was reported that growth factors and extracellular matrix components are found to be important in the control of the transdifferentiation process of vertebrate pigmented epithelial cells [15].

In embryonic rat, RPE cells were seen to transdifferentiate into neural retina [11], although this ability was restricted until age E13 [14]. These findings show that environmental factors regulate the expression of neuron-associated genes in RPE cells.

To evaluate the potential of adult mammalian RPE cells for transdifferentiation into neural cells, we used two adult human RPE cell lines. ARPE-19 was harvested from the eye of a 19-year-old young adult. H80HrPE-6 is one of the cell lines which we isolated from an 80-year-old person, and the cells were cloned from a single cell. The original primary cells were shown to be capable of transdifferentiating into lens. It was reported that H80HrPE-6 cells became lens cells when they were cultured in hard agar and the other on MATRIGEL which contained growth factors, and this cell line expressed key genes of lens development and regeneration, Pax-6 and six-3 [15,17].

ARPE-19 is a retinal pigment epithelial (RPE) cell line derived in 1986 by Amy Aotaki-Keen from the normal eyes of a 19-year-old male. This cell line has been widely used in studies of RPE function as well as gene expression [18].

Under light microscopy, both ARPE-19 and H80HrPE-6 showed flat and polygonal epithelial shapes in medium with FBS. They proliferated constantly and could be passaged with the aid of trypsin EDTA. Cells of both lines remained healthy after freeze-and-thaw manipulation. These were common characteristics of the two cell lines. While neither cell line showed pigmentation during short-time culture, ARPE-19 cells contained pigment after several months' culture.

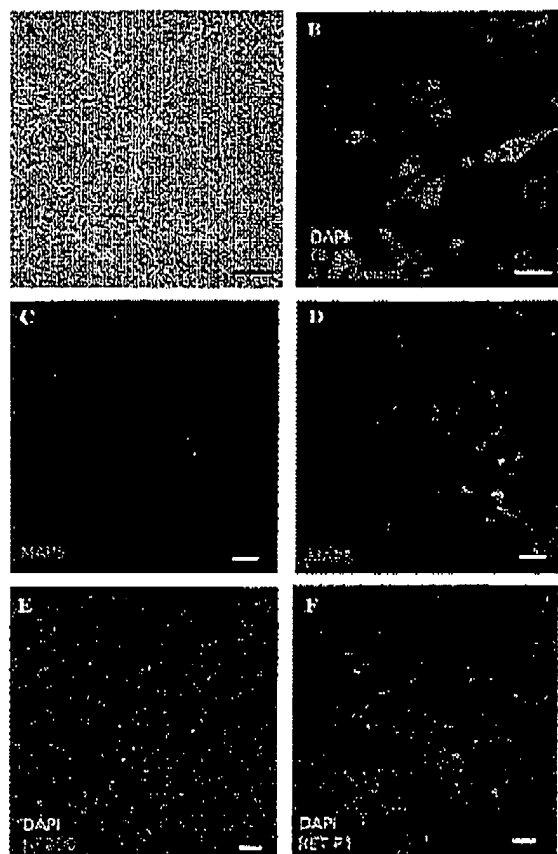


Fig. 2. (A) Phase contrast photograph of RPE cells cultured in serum free DMEM/T12 with bFGF and EGF on laminin/poly-L-ornithine-coated dishes. The bar = 100 μm. (B–F) RPE cells after induction of differentiation with retinoic acid without bFGF. Nuclei were stained with DAPI. (B) Immunocytochemistry for β-III tubulin (green) and GFAP (red). The bar = 50 μm. (C,D) Immunocytochemistry for MAP5 (green) in ARPE-19 (C) and H80HrPE-6 (D). The bar = 100 μm. (E) Immunocytochemistry for neurofilament 200K (green). The bar = 100 μm. (F) Immunocytochemistry for rod-opsin (RET PI) (green). The bar = 100 μm.

In a previous study, it was reported that once RPE cells were moved from an *in vivo* to an *in vitro* culture system, they began to express the class III isotype of β-tubulin [19], which is normally restricted to neuronal cell phenotypes in the retina [20,21]. In the same study, all the cells of both lines expressed β-III tubulin in a medium in which pigment epithelial cells can proliferate. From this result it appears that the RPE cells are closely related to neuronal lineage cells and easily acquire their characteristics *in vitro*.

Palmer et al. succeeded in isolating the neural stem cells from adult rat hippocampus tissue on laminin/poly-

L-ornithine coated dishes with a bFGF containing medium. To induce differentiation, the cells were cultured in a medium with 0.5% serum and 500 nM all-trans retinoic acid [22,23]. We used the same methods for the RPE transdifferentiation by culturing the RPE cell lines on laminin/poly-L-ornithine coated dishes and in a medium with bFGF and EGF. After culturing under stem cell maintenance conditions, the shape of most cells changed to spherical or spindle-like, and the cells no longer expressed the RPE marker or ZO-1.

After the cells were cultured in the medium with retinoic acid to induce neural differentiation, some of them extended processes and not only β-III tubulin—but also MAP5-positive cells were detected. A few cells expressed neurofilament 200, which is a mature neuronal marker. These adult human RPE cells therefore have the potential to differentiate into mature neurons. The ratio of MAP5-positive cells was much higher in ARPE-19 than in H80HrPE-6, which means the younger RPE cells could more easily differentiate into neurons. However, it is remarkable that the RPE cells from an 80-year-old human could still maintain the ability to differentiate into neurons.

Another interesting point is that we did not observe any GFAP-positive cells even with 10% fetal bovine serum in culture medium. Neural stem cells usually differentiate into only glia with this high concentration of serum (unpublished data). Together with the fact that all of the cells were positive for β-III tubulin, it can be postulated that these dedifferentiated RPE cells attained the characteristics of neuronal progenitor like cells, so that they could not differentiate into glial cells. Moreover, it appears that we could not use β-III tubulin as a mature neuronal marker because the cells with a flat, epithelial-like morphology also proved to be immunoreactive for it in this study. We therefore consider β-III tubulin to be a neuronal lineage marker.

In summary, we showed that adult human retinal pigment epithelium cells are capable of differentiating into neurons. The ability to differentiate was greater in the young than in the old human RPE cell line, although the latter could still differentiate. Since we could not observe any photoreceptor marker or glial marker positive cells, conditions making it possible to obtain retinal neuronal cells from RPE cell lines should be examined further.

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